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(54) Title: STAGING OF PROSTATE CANCER

(57) Abstract

The invention relates to a method for staging of prostate cancer, i.e. differentiating organ confined prostate cancer (PCa) from non-organ confined PCa in a patient, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) and optionally also prostate specific antigen (PSA) have been determined. In the method, hK2 is used as a marker distinguishing patients with organ confined PCa from patients with non-organ confined PCa. Moreover, the invention relates to a method for grading of prostate cancer, i.e. differentiating patients with aggressively progressing prostate cancer (PCa) from patients with less aggressively progressing PCa, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) has been determined. In the method, hK2 alone is used as the marker.

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STAGING OF PROSTATE CANCER

FIELD OF THE INVENTION

This invention relates to a method for differentiating of organ confined prostate cancer (PCa) from non-organ confined PCa in a patient, i.e. for staging of PCa in a patient. Furthermore, this invention concerns a method for differentiating patients with aggressively progressive PCa from patients with less aggressively progressive PCa, i.e. for preoperative grading of PCa in a patient.

INTRODUCTION AND BACKGROUND

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

Prostate cancer (PCa) is the most commonly diagnosed cancer in men, and death rates for PCa are second only to those for lung neoplasms[1]. With the emergence of prostate-specific antigen (PSA) in 1971[2] and its introduction into clinical use in 1979[3], prostate-specific antigen has emerged as the most important tumor marker in the urologic speciality[4,5]. By far the most valuable field of its clinical application is the postoperative follow-up after radical prostatectomy where - due to an organ specificity that is sufficient for all practical purposes[6] - evidence of recurrent disease can be based solely on the re-emergence of PSA in serum[6-11].

Despite its limitations due to lacking sensitivitiy and specificity for prostate cancer[5,9,12,13] its use is also established in the diagnosis of prostate cancer. The lack of sensitivity and specificity however lead to the creation of various parameters based on PSA to enhance the clinical

utility of this tumor marker, among others PSA-velocity[14], PSA-density[15], transition zone PSA-densitiy[16], age-specific PSA-ranges[17] and the ratio of free to total PSA (%fPSA)[18,19,20].

The application of PSA in the preoperative staging of prostate cancer has demonstrated, that serum PSA levels correlate with tumor volume, advancing clinical and pathological stage[7,9,13]. On the other hand, it has been shown that, on an individual basis, single PSA-levels are not specific enough to permit precise prediction of final pathological stage[11,12]. The most effective approach in the treatment of prostate cancer can be performed, when the tumor is still organ confined. Information can be obtained from the results of systematic sextant biopsies. However, a serum marker for the more accurate staging of prostate would yield to important and more easily available new information.

Presently, a new serum marker of prostatic origin, human glandular kallikrein 2 (hK2), a closely related protein of the same enzymatic family, the so-called serin proteases emerges as a potential marker for prostate tissue of benign and malignant differentiation[21,22,23]. The gene for hK2 is 80% homologous to the PSA-gene. Recent studies indicated that both hK2 and PSA-mRNA´s are found exclusively in the prostatic epithelium[24,25,26]. They also share the feature of androgen-controlled expression[25,27]. Finally, hK2 is the enzyme that cleaves pro-PSA and thus activates it into its enzymatically active form[28]. The less common term of human glandular kallikrein 3 for prostate-specific antigen underlines the relationship of both enzymes.

From a biochemical point of view, the close homology of both proteins made the design of specific monoclonal antibodies necessary, that possibly do not cross-react with the other kallikrein. A recent epitope mapping study of hK2

and PSA[29,30] showed various degrees of cross-reactivity of anti-PSA antibodies with hK2[31]. Based on this information, immunoassays of different designs were created for the specific measurement of hK2[29].

Clinically, serum concentrations of hK2 have been used in an attempt to improve the detection of prostate cancer in patients with a total PSA of 4-10 ng/ml, (the diagnostic gray zone)[32], as well as measurement of cytoplasmatic expression of hK2 (and PSA) in radical prostatectomy specimen[22].

Clinically, understaging of prostate tumors is a major problem when selecting treatments of curative intention. Of clinically organ confined prostate tumors undergoing radical prostatectomy, 26%–43% show extracapsular disease[35,36]. Of these 30%-80% of them develop into advanced disease within 10 years[37,38]. Thus, there is a clear need for new diagnostic tools to reduce this understaging. In addition to the unsatisfactory clinical T-staging, prostate cancer prognosis depends also on histological grading of the tumor[38,39] and this is frequently unreliable when performed preoperatively on sextant biopsies. In addition, increases in serum PSA values do not adequately reflect the more advanced pathological grade, especially not in the intermediate range of PSA[12,40,41].

OBJECTS AND SUMMARY OF THE INVENTION

One object of the present invention is to provide a method for staging PCa in a patient.

In a first study we focused our attention to serum concentrations of hK2 in 68 radical prostatectomy patients to evaluate if hK2-concentrations are different in various pathologic stages and, moreover, if hK2 concentrations are different in patients with organ confined and non-organ

confined prostate cancer. Since PSA cannot reliably predict an organ confined cancer for an individual patient[11,12], this capability might be an important feature of this new serum marker in the preoperative biochemical staging of adenocarcinoma of the prostate.

Another object of the present invention is to provide a method for assessing the grade of PCa in a patient.

Human glandular kallikrein (hK2) possesses 80% structure identity with prostate-specific antigen (PSA) and is secreted by identical prostate epithelial cells. Although increasing with pathological stage, serum PSA is not clinically useful in assessing the aggressiveness of prostate cancer in individual cases. A study was carried out in order to assess whether hK2 as such allows for differentiation of well, moderately and poorly differentiated prostate cancer cells, compared to different PSA forms.

Thus, according to one aspect this invention relates to a method for staging of prostate cancer, i.e. differentiating organ confined PCa from non-organ confined PCa in a patient, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) and optionally also prostate specific antigen (PSA) have been determined. According to the invention, hK2 is used as a marker distinguishing patients with organ confined PCa from patients with non-organ confined PCa.

According to another aspect, the invention relates to a method for grading of prostate cancer, i.e. differentiating patients with aggressively progressing PCa from patients with less aggressively progressing PCa, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) has been determined. According to the invention, hK2 alone is used as the marker.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a to 1c show concentrations of hK2, the [hK2]*[total PSA]/[free PSA] algorithm and for total PSA for organ confined (oc) and non-organ confined (noc) cancers.

DETAILED DESCRIPTION OF THE INVENTION

According to a preferable embodiment, hK2 alone is used as the marker in the staging method. Combinations of hK2 and PSA, wherein PSA means the free PSA, the complexed PSA or the total PSA, can alternatively be used as the marker. In the latter case, the marker is preferably the algorithm hK2 x total PSA/free PSA.

The grading method is preferably either i) a discrimination of patients with well and moderately differentiated PCa on the one hand, and poorly differentiated PCa on the other hand, or ii) a discrimination of patients with moderately differentiated PCa on the one hand, and poorly differentiated PCa on the other hand.

The grading method is particularly useful in patients having a total PSA in the range 1 to 20 ng/ml, especially in the range 3 to 15 ng/ml.

The detection of PSA in serum in its complexed and non-complexed forms has become an established part of the pre- and post-therapeutic evaluation of prostate cancer. Once the histological diagnosis of prostate cancer is done, and evaluation of the patient suggests a clinically localized prostate carcinoma, radical retropubic prostatectomy is the treatment standard to be applied in a curative attempt for the patient. Several studies were able to show that 40 to 60% of patients however had a capsular penetration of the cancer. Prognosis -particularly in terms of PSA-free survival - is closely related to pathological stage.

Patients with a pathologically organ confined cancer achieve PSA-free survival rates and hence possible cure rates of >90% over a period of up to 10 years, whereas capsular penetration (pathological stage T3a or greater) markedly reduces PSA-free survival and cure rates. An important part in the evaluation is the analysis of the prostatic biopsies for Gleason grade in combination with transrectal ultrasound and digital rectal examination.

On the biochemical side of prostate cancer evaluation, it has been shown, however, that PSA - neither alone, nor in any combination, (e.g. %fPSA, free PSA, complexed PSA, etc.) - is reliable enough to predict on an individual basis the pathological stage of clinically localized prostate cancer. Therefore a biochemical marker that might add information on the pathological staging on an individual basis would enable the clinician to more carefully select those patients who will have the highest chance to benefit from radical prostatectomy, i.e. to be cured from prostate cancer.

As a potential new marker, much attention has recently been attached to the detection of the human glandular kallikrein 2 (hK2) in serum and in prostate tissue specimens. In our study we aimed to investigate the ability of serum levels of hK2 to discriminate patients with PCa with pathologic stages 2a/b -organ-confined cancers- from those with extraprostatic extension (pathological stages $\geq$3a). Serum sampling and rapid processing of samples without prior manipulation of the prostate one day prior to surgery provided evidence for optimal analytical conditions, pathological examination of the prostatectomy specimens according to the Stanford-protocol for accurate pathological staging.

Simple tests are greatly needed in order to reliably estimate the aggressiveness of a prostate cancer and to provide more accurate information prior to any therapeutic

decisions (e.g. radical prostatectomy, radiotherapy or watchful waiting) to avoid over- or undertreatment. PSA has been established as an important prognostic parameter. However, serum PSA values do not adequately describe the advancing pathological grade[12]. Because serum PSA production tend to decrease with increasing histological grade (dedifferentiation), also in the low range of clinically localised disease, total PSA has been a poor predictor of prostate cancer prognosis[6]. Only at highly elevated levels (e.g. greater than 100 ng/ml) PSA clearly indicates the presence of advanced metastatic disease.

This is the first detailed clinical report documenting hK2 as a more adequate tumor marker to reflect the dedifferentiation of prostatic tumor cells. Of special clinical importance is the capability of hK2 to identify poorly differentiated tumors better than PSA in the intermediate range between 3 to 15 ng/ml PSA. One of the main topics in therapy decision-making today is, on one hand, to exclude tumors with the high risk of extracapsular disease (particularly with seminal vesical extension) from local therapy in curative intention. On the other hand overtreatment in relatively non-aggressive disease cases should be avoided as the data from the natural history of prostate cancer show.

Our results shown below are supported by the observations of Darson et al. demonstrating a more dramatic increase in immunohistochemical staining for hK2 with increasing histological grade compared to PSA[22]. In addition there are indications that RT-PCR of hK2 may predict final positive lymph node states[45].

EXPERIMENTAL SECTION

Study I

The aim of the first study was to evaluate the use of hK2 or hK2 in combination with forms of PSA as markers for staging of PCa in a patient.

In the first study, we investigated serum levels of human glandular kallikrein 2 (hK2) in patients with prostate cancer treated with radical retropubic prostatectomy to investigate wether preoperative serum concentrations of hK2 were different in patients with pathological stage 2a/b cancers compared to those with a pathological stage $\geq$3a disease and as such could be helpful in the preoperative prediction of organ confined cancers versus extraprostatic extension of the tumor.

Materials and methods

Patient selection and evaluation:

Serum samples of 68 men scheduled for radical retropubic prostatectomy for clinically localized prostate cancer were collected one day prior to surgery. No patients received hormonal treatment before surgery. Serum was collected prior to any manipulation of the prostate and brought to our laboratory, where it was stored at -80°C until analysis.

Histological characterisation of the prostate:

The prostate was prepared according to the Stanford protocol[33]. It was inked over the entire surface, fixed in formalin for at least 24 hours, and processed with a 3-mm step-section technique. The Gleason system was used for histologic grading[34], and staging was according to the second revision of the fourth edition of the TNM

classification.

Detection of hK2:

For the detection of hK2 we used a three-step immunofluorometric assay described earlier. In short, a first antibody that does not cross react with hK2 is given in excess to prevent free and total PSA to react in further reaction steps. Then, a second, biotinylated antibody is added, that reacts with hK2 only because the corresponding epitope on PSA has been blocked in the first step, and binds it to the streptavidin coated microtitration well. All PSA is removed by washing. In the third step, a Europium-labeled antibody reacts with the immobilized hK2. Europium forms a fluorescent chelate, that is proportional to the amount of hK2. Immunological crossreaction measured with recombinant PSA was less than 0.1%. Analytical detection limit, defined the 3xSD imprecision of the zero calibrator, was 0.01 ng/ml, whereas the functional detection limit was 0.03 ng/ml, defined as level at which interassay coefficient of variation (CV) was below 20%.

Detection of total and free PSA and %fPSA:

To detect total PSA and free PSA, we used the DELFIA ProStatus Dual PSA-Total/free assay. The assay works on a sandwich-based technique. In the first step, free and total PSA are equimolarly bound to a solid phase anti-total PSA antibody. In the next step, Europium-labeled antibodies bind to an antigenic site that is accessible only in free PSA-molecules. Simultaneously, Samarium-labelled antibodies bind to antigenic sites that are accessible to both free and total PSA. Both lanthanides form fluorescent chelates, that are proportional to the amount of free (Europium only) and total (Samarium) PSA. From both results, the ratio of free to total PSA (%fPSA) is calculated.

Algorithm using hK2, total and free PSA:

For the clinical analyses, we investigated three algorithms that aimed to combine hK2 and free and total PSA: First the concentration of hK2 times total PSA divided by free PSA, second the concentration of hK2 divided by the concentration of total PSA and third the concentration of hK2 divided by the concentration of free PSA. Analysis of the three algorithms showed, that the first one - [hK2]*[total PSA]/[free PSA]- was the most powerful algorithm in the discrimination of organ-confined and non-organ confined cancers, hence in our further analysis, this algorithm was applied and the latter two algorithms were neglected.

Study design and statistical workup of data:

For each of the analytes (hK2, total PSA, free PSA), for %fPSA and for the [hK2]*[total PSA]/[free PSA] algorithm, means, ranges and standard errors were calculated. Means and ranges were compared in patients with organ-confined and non-organ-confined tumors. Calculation of significance of the differences was performed using Mann-Whitney U-test. A p value of 0.05 or less was considered significant. hK2 and [hK2]*[total PSA]/[free PSA] results were obtained, the results were then compared to total PSA, free PSA and %fPSA. Box plots (Figures 1a-1c) visualize concentrations of hK2, the [hK2]*[total PSA]/[free PSA] algorithm and for total PSA for organ and non-organ confined cancers.

Results of Study I

Of 68 patients operated, 38 patients had organ confined cancers, workup of 30 men showed non-organ confined cancers. Summary Table 1 shows means and p-values of the examined analytes and algorithms, which briefly tell that hK2 alone and the [hK2]*[total PSA]/[free PSA] algorithm gave the most accurate information, followed by total PSA,

free PSA and the non-significant %fPSA in the discrimination of organ-confined vs. non-organ confined cancers.

hK2 was undetecable in at levels <0.03 ng/ml in 5/38 patients (= 13.1%) with organ-confined cancers and in 0/30 patients with non-organ confined cancers. Mean hK2 concentration of all samples was 0.18 ng/ml (range: <0.03-0.94 ng/ml). In organ confined cancers, mean hK2 concentration was 0.09 ng/ml, with a range of <0.03-0.67 ng/ml. In non-organ-confined cancers, mean hK2 concentration was 0.30 ng/ml (range 0.04-0.94 ng/ml). Complete data split by pathological stage are shown in Table 2, and the box plot of hK2 concentrations is shown in Figure 1a. Mann-Whithey U-test revealed a statistically highly significant difference for hK2 concentration in organ-confined vs. non-organ-confined cancers ($p= 0.0001$).

Mean results of the [hK2]*[total PSA]/[free PSA] algorithm was 1.54 (range: 0.06 - 10.16). In organ confined cancers, mean value was 0.93 with a range of 0.06 - 5.79. In non-organ-confined cancers, mean results were 2.31 (range 0.30 - 10.16). Complete data split by pathological stage are shown in Table 3, and the box plot of [hK2]*[total PSA]/[free PSA] results is shown in Figure 1b. Mann-Whithey U-test revealed a statistically highly significant difference in organ-confined vs. non-organ-confined cancers ($p= 0.0005$).

Total PSA concentration of all samples was 10.73 ng/ml (range: 3.34 - 62.3 ng/ml). In organ confined cancers, mean PSA concentration was 7.5 ng/ml, with a range of 3.34 - 24.1 ng/ml. In non-organ-confined cancers, mean PSA concentration was 14.81 ng/ml (range 3.43 - 62.3 ng/ml). Complete data split by pathological stage are shown in Table 4, and the box plot of total PSA results is shown in Figure 1c. Mann-Whitney U-test showed a statistically significant difference for PSA concentration in organ-

confined vs. non-organ-confined cancers (p= 0.0023).

Results of free PSA and %fPSA are shown in Table 1. Due to the superior results that were found using only hK2, the [hK2]*[total PSA]/[free PSA] algorithm and total PSA, we refrained from showing more detailed tables and box plots for free PSA and %fPSA.

Our results in the detection of hK2 give rise to several conclusions. First we were able to show, that hK2 can be detected in the vast majority of patients (63/68) with clinically localised prostate cancer. On the other hand, the existence of 5 patients with serum levels below the detection limit makes the need for an assay with an even lower functional detection obvious, particularly for the evaluation of those prostate cancer patients that were hK2-negative in our cohort, but also for patients without prostate cancer. The second point is, that no patient with a non-organ confined cancer had undetectable hK2-concentration.

The improved hK2 assay based on monoclonal anti-PSA antibodies had a cross-reactivity with PSA of less than 0.1%. That value was sufficiently low to allow us to evaluate the clinical significance of specific measurement of hK2 in serum, where the median hK2 level corresponded to approximately 1.3-1.6% of the PSA concentration. Despite the low functional sensitivity limit of 0.05 ng/ml (defined from the coefficient of variation, less than 20%), the assay did not detect hK2 immunoreactivity in the following subjects: all healthy controls, 50% of the men with BPH, 30% of the patients with clinically localized PCa and 4% of those with clinically advanced PCa. Clearly, in particular to appropriately evaluate subjects without malignant prostatic lesions, we assigned an hK2 level of 0.04 ng/ml to all samples with no detectable hK2 immunoreactivity. This proved to be necessary to avoid introducing any unsuitable distinction between the men with BPH and the

cancer patients with hK2 levels below 0.05 ng/ml. That conclusion is supported by results we obtained by using a value of 0 for all undetected hK2 levels, or by excluding patients with hK2 concentrations below the detection limit (results not shown).

In our study the inclusion of hK2 in the diagnostic preoperative workup of radical prostatectomy patients improved separation of organ-confined and non-organ confined cancers. Either hK2 alone or the algorithm [hK2]*[total PSA]/[free PSA] was of statistical superiority for this purpose as compared to total PSA, free PSA and the relation of free to total PSA (%fPSA). As such, the inclusion of hK2 in the preoperative biochemical evaluation of prostate cancer might be a useful tool for an improved selection of patients with histologically proven prostate cancer.

Study II

The aim of the second study was to evaluate the use of hK2 measurement as such for grading of PCa in a patient.

Materials and Methods

The study population consisted of 122 consecutive patients with histologically proven prostate cancer. The histologically based diagnosis was performed on tissues obtained from transrectal ultrasound guided sextant biopsies of the prostate and/or from the whole gland obtained after radical prostatectomy. The grades were classified as well (G1,n=35), moderately (G2,n=61) or poorly (G3,n=26) differentiated carcinoma. The patients had not previously been subjected to anti-androgenic treatment, transurethral resection, radical prostatectomy or radiotherapy.

Blood samples were obtained before any prostatic

manipulation. After clot formation the samples were centrifuged and serum was collected and frozen at $-70^{0}C$. The samples were thawed immediately before measurement. hK2 measurement was done by an indirect immunofluorometic assay previously described[41] and based on an indirect PSA scavenger step. The analytical sensitivity (background +3SD) was 0.01 ng/ml and the functional sensitivity 0.05 ng/ml (defined as an intra-assay coefficient of variations of 20% or less) (when 25 μl serum aliquots were used) or 0.02-0.03 ng/ml (when 50 μl serum aliquots were used). The cross reactivity of the assay with PSA amounted to less than 0.1% by the use of two scavenger antibodies to prevent PSA from being sandwiched in the assay.

Total and free PSA were determined by the commercially available monoclonal immunofluorometric Delfia ProStatus PSA Free/Total kit (Wallac Oy, Turku, Finland)[42].

PSA bound to α-1-antichymotrypsin (PSA ACT) was also measured by an immunofluorometric assay similar to that of the one previously described[43], except that anti PSA monoclonal IgG (H117) was used as the capture antibody and Eu-labeled anti-ACT monoclonal IgG (241) as the detection antibody.

Statistical analysis:

Multivariate logistic regression analysis was performed to detect the best combinations of the tumor markers. Statistical analysis was done using commercially available computer software. Within each group of tumor differentiation, the median and mean levels (±SD) of total PSA, free PSA, PSA-ACT and hK2 were calculated. Same procedure was done for following combinations: free/total PSA; hK2/free PSA; (hK2/free PSA)×(total PSA/free PSA); free PSA/PSA-ACT; PSA-ACT/total PSA; hK2/total PSA and free PSA/(total PSA×hK2). We used the non-parametric Mann-Whitney U Test to determine the statistical significance of

the differences between the groups.

For all analyses a p value of <0.05 was considered statistically significant.

Results of Study II

The descriptive statistics of the different markers and their combinations in G1, G2 and G3 tumors (median, mean ±SD) are shown in Table 5. Total PSA increased about 2-fold from G1 to G2(p=0.0002) and from G2 to G3 tumors (13.1 vs.26.5 ng/ml). The latter increase was however not significant (p=0.18). In contrast hK2 also increased from G2 to G3 with a factor of 3 (p=0.02). The free to total PSA ratio was decreased in G1 compared to G2 (0.15 vs. 010, p=0.007). No statistically significant difference was found between the G2 to G3 groups (0.10 vs. 0.11, p=0.93). However, the hK2/free PSA ratio also distinguished between G2 and G3 tumors (0.11 vs. 0.22, p=0.002). In multivariate regression analysis, the combinations containing hK2 ((hK2/F)×(T/F); hK2/T; F/(T×hK2)) also differentiated between G2 and G3 in a statistically significant manner. Results from G1 vs. G3 are listed in Table 5.

Table 6 compares the median for the combination (G1+G2) of well and moderately differentiated prostate cancers to the poorly differentiated tumors (G3). The statistically most significant differences between these groups were obtained by hK2 (p=0.001), hK2/free PSA (p=0.0003) and free PSA/(T×hK2) (p=0.0004). A p value of 0.01 for total PSA was recorded whereas the free to total PSA ratio failed to differentiate between the two groups.

From a clinical point of view, cancers in the total PSA range of 3-15 ng/ml form the most important group. In this range, poorly differentiated carcinoma could not be distinguished from G1/G2 tumors by total PSA (10.6 vs. 7.8 ng/ml, p=0.20), free PSA (1.19 vs. 0.85 ng/ml, p=0.55) or

PSA-ACT (9.3 vs. 7.3 ng/ml, p=0.22) (Table 7). In contrast, hK2 increased 2.9-fold from 0.08 ng/ml (G1/G2) to 0.23 ng/ml for the poorly differentiated G3-tumors (p=0.03). Furthermore, the ratio hK2/free PSA distinguished between G1/G2 and G3 carcinomas (0.09 vs. 0.17, p=0.02), whereas percent free PSA/total PSA failed to do so (0.12 vs. 0.10, p=0.3).

The results show that hK2 significantly improved the identification of the more aggressive (G2 to G3) tumors, compared to total, free PSA and PSA-ACT. Important is the observation, that the improved detection of aggressiveness was also seen within the intermediate range of total PSA (3-15 ng/ml). Thus, hK2 as such is a useful tool for pretreatment decision analysis.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

Tab. 1 Summary table of means of the examined analytes and algorithms showing results and p-values in the discrimination of organ-confined (oc) and non-organ confined (noc) cancers

	mean	oc	noc	p-value#
HK2 (ng/ml)	0.18	0.09	0.30	0.0001
HK2* total PSA/ free PSA	1.54	0.93	2.31	0.0005
total PSA (ng/ml)	10.73	7.50	14.81	0.0023
free PSA (ng/ml)	1.37	0.86	2.03	0.0037
%fPSA	13.10	12.51	13.85	0.6745

= Mann-Whitney-U analysis

Tab. 2: Distribution of HK2-concentrations split by pathological stages and lymph node status. Subdivision shows values for organ-confined (oc) and non-organ-confined (noc) cancers (ng/ml)

	Mean	Std. Error	Count	Minimum	Maximum
pT2a	0.05	0.02	5	<0.03	0.11
pT2b	0.10	0.02	33	<0.03	0.67
oc	**0.09**	**0.02**	**38**	**<0.03**	**0.67**
pT3a	0.24	0.06	21	0.04	0.94
pT3b	0.14	0.00	2	0.14	0.14
pT4a	0.52	0.15	4	0.13	0.83
LN-pos	0.47	0.05	3	0.37	0.53
noc	**0.30**	**0.05**	**30**	**0.04**	**0.94**
total	**0.18**	**0.03**	**68**	**<0.03**	**0.94**

Tab. 3: Distribution of (HK2* total PSA/free PSA) results split by pathological stages and lymph node status. Subdivision shows values for organ-confined (oc) and non-organ-confined (noc) cancers

	Mean	Std. Error	Count	Minimum	Maximum
pT2a	0.42	0.21	5	0.06	1.26
pT2b	1.00	0.20	33	0.10	5.79
oc	**0.93**	**0.18**	**38**	**0.06**	**5.79**
pT3a	2.20	0.57	21	0.30	10.16
pT3b	1.18	0.06	2	1.12	1.24
pT4a	2.60	0.93	4	0.95	5.18
LN-pos	3.41	0.97	3	1.68	5.03
noc	**2.31**	**0.43**	**30**	**0.30**	**10.16**
total	**1.54**	**0.23**	**68**	**0.06**	**10.16**

Tab. 4: Distribution of total PSA-concentrations split by pathological stages and lymph node status. Subdivision shows values for organ-confined (oc) and non-organ-confined (noc) cancers (ng/ml)

	Mean	Std. Error	Count	Minimum	Maximum
pT2a	6.63	1.11	5	3.93	9.54
pT2b	7.64	0.76	33	3.34	24.10
oc	**7.50**	**0.67**	**38**	**3.34**	**24.10**
pT3a	12.57	2.25	21	3.43	42.50
pT3b	10.00	5.24	2	4.76	15.24
pT4a	25.33	12.49	4	7.53	62.30
LN-pos	19.67	3.69	3	14.20	26.70
noc	**14.81**	**2.35**	**30**	**3.43**	**62.30**
total	**10.73**	**1.18**	**68**	**3.34**	**62.30**

Table 5 Descriptive statistics and statistical significance of differences between each tumor grade (all patient cases)

	G1 (n=35)		G2 (n=61)		G3 (n=26)		G1 vs G2	G1 vs G3	G2 vs G3
	Median	Mean+-SD	Median	Mean+-SD	Median	Mean+-SD	p-value	p-value	p-value
ALTER	71.96	72.01+-8.9	70.67	68.52+-8.6	71.92	70.54+-7.5	0.11	0.62	0.43
PSA-T	6.62	9.94+-13.1	13.10	119.23+-323.5	26.50	92.80+-130.9	**0.0002**	**0.0002**	0.18
PSA-F	0.83	1.56+-2.4	1.48	17.35+-66.4	2.30	14.19+-22.8	**0.01**	**0.003**	0.23
PSA-ACT	5.19	9.10+-12.0	12.27	112.84+-300.6	26.95	69.58+-99.1	**0.0001**	**0.0001**	0.32
hK2	0.068	0.238+-0.6	0.140	3.084+-13.4	0.430	9.106+-20.7	**0.002**	**0.0001**	**0.02**
F/T	0.149	0.158+-0.052	0.105	0.122+-0.061	0.108	0.134+-0.085	**0.0007**	**0.04**	0.93
hK2/F	0.085	0.104+-0.062	0.109	0.141+-0.100	0.219	0.444+-0.600	**0.04**	**0.0002**	**0.002**
(hK2/F)x(T/F)	0.595	0.773+-0.660	1.047	1.293+-0.917	1.744	4.229+-5.839	**0.001**	**0.0001**	**0.01**
F/ACT	0.175	0.179+-0.072	0.109	0.136+-0.083	0.135	0.190+-0.182	**0.001**	0.17	0.40
ACT/T	0.901	0.907+-0.100	0.932	0.938+-0.095	0.853	0.843+-0.179	**0.05**	0.27	**0.01**
hK2/T	0.013	0.016+-0.009	0.011	0.019+-0.018	0.028	0.061+-0.087	0.69	**0.01**	**0.01**
F/(T*hK2)	2.484	3.093+-2.775	0.615	1.020+-1.203	0.199	0.533+-0.938	**0.00005**	**0.000006**	**0.01**

Table 6 Descriptive statistics and statistical significance of differences between combined tumor grade G1 and G2 versus grade G3 group (all patient cases)

	G1+G2 (n=96)		G3 (n=26)		G1+2 vs G3
	Median	Mean+-SD	Median	Mean+-SD	p-value
ALTER	70.72	69.70+-8.8	71.92	70.54+-7.5	0.73
PSA-T	10.20	82.39+-267.8	26.50	92.80+-130.9	**0.01**
PSA-F	1.10	12.03+-54.4	2.30	14.19+-22.8	**0.05**
PSA-ACT	9.11	77.87+-249.1	26.95	69.58+-99.1	**0.03**
hK2	0.120	2.125+-10.9	0.430	9.106+-20.7	**0.001**
F/T	0.121	0.134+-0.060	0.108	0.134+-0.085	0.45
hK2/F	0.099	0.129+-0.090	0.219	0.444+-0.600	**0.0003**
(hK2/F)x(T/F)	0.957	1.118+-0.871	1.744	4.229+-5.839	**0.001**
F/ACT	0.125	0.151+-0.081	0.135	0.190+-0.182	0.97
ACT/T	0.924	0.928+-0.097	0.853	0.843+-0.179	**0.03**
hK2/T	0.012	0.018+-0.015	0.028	0.061+-0.087	**0.005**
F/(T*hK2)	0.923	1.719+-2.113	0.199	0.533+-0.938	**0.0004**

Table 7 Descriptive statistics and statistical significance of differences between combined tumor grade G1 and G2 versus grade G3 group, and between each tumor grade, respectively, (patient cases with total PSA in the range 3 - 15 nl/ml))

	G1+G2 (n=47)		G3 (n=8)		G1+2 vs G3
	Median	Mean+-SD	Median	Mean+-SD	p-value
ALTER	70.6	69.0+-8.3	68.5	66.9+-7.1	0.50
PSA-T	7.69	7.80+-3.01	10.06	9.40+-3.28	0.20
PSA-F	0.85	0.98+-0.46	1.20	1.14+-0.65	0.55
PSA-ACT	7.24	7.30+-3.19	9.38	8.48+-2.71	0.22
hK2	0.075	0.109+-0.085	0.230	0.208+-0.127	**0.03**
F/T	0.114	0.132+-0.054	0.100	0.119+-0.068	0.36
hK2/F	0.092	0.114+-0.073	0.174	0.231+-0.206	**0.02**
(hK2/F)x(T/F)	0.959	0.996+-0.652	1.346	3.218+-5.151	0.08
F/ACT	0.124	0.147+-0.071	0.116	0.137+-0.092	0.46
ACT/T	0.922	0.923+-0.094	0.906	0.924+-0.122	0.78
hK2/T	0.011	0.015+-0.014	0.027	0.023+-0.012	**0.04**
F/(T*hK2)	1.298	1.954+-1.859	0.513	0.881+-0.880	**0.01**

	G1 (n=20)		G2 (n=27)		G3 (n=8)		G1 vs G2	G1 vs G3	G2 vs G3
	Median	Mean+-SD	Median	Mean+-SD	Median	Mean+-SD	p-value	p-value	p-value
ALTER	72.5	72.0+-7.5	68.9	67.0+-8.3	68.5	66.9+-7.1	0.06	0.18	0.92
PSA-T	7.59	7.79+-3.38	7.83	7.81+-2.81	10.06	9.40+-3.28	0.88	0.40	0.17
PSA-F	1.01	1.15+-0.54	0.82	0.86+-0.37	1.20	1.14+-0.65	0.09	0.89	0.30
PSA-ACT	7.00	7.19+-3.58	7.30	7.36+-2.98	9.38	8.48+-2.71	0.69	0.22	0.30
hK2	0.090	0.107+-0.079	0.075	0.110+-0.090	0.230	0.208+-0.127	0.81	0.09	**0.03**
F/T	0.137	0.152+-0.051	0.105	0.118+-0.052	0.100	0.119+-0.068	**0.02**	0.10	0.77
hK2/F	0.079	0.090+-0.048	0.103	0.130+-0.083	0.174	0.231+-0.206	**0.05**	**0.01**	0.07
(hK2/F)x(T/F)	0.588	0.675+-0.437	1.062	1.210+-0.690	1.346	3.218+-5.151	**0.01**	**0.01**	0.34
F/ACT	0.161	0.172+-0.074	0.109	0.131+-0.066	0.116	0.137+-0.092	**0.05**	0.18	0.83
ACT/T	0.902	0.915+-0.114	0.922	0.929+-0.079	0.906	0.924+-0.122	0.28	0.94	0.60
hK2/T	0.012	0.013+-0.006	0.010	0.017+-0.018	0.027	0.023+-0.012	0.90	**0.04**	0.09
F/(T*hK2)	1.651	2.597+-2.550	1.173	1.526+-1.059	0.513	0.881+-0.880	0.15	**0.01**	**0.02**

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CLAIMS

1. A method for staging of prostate cancer, i.e. differentiating organ confined prostate cancer (PCa) from non-organ confined PCa in a patient, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) and optionally also prostate specific antigen (PSA) have been determined, <u>characterized</u> in that hK2 is used as a marker distinguishing patients with organ confined PCa from patients with non-organ confined PCa.

2. The method according to claim 1, <u>characterized</u> in that hK2 alone is used as the marker.

3. The method according to claim 1, <u>characterized</u> in that a combination of hK2 and PSA, wherein PSA means the free PSA, the complexed PSA or the total PSA, is used as the marker.

4. The method according to claim 3, <u>characterized</u> in that the marker is the algorithm hK2 x total PSA/free PSA.

5. A method for grading of prostate cancer, i.e. differentiating patients with aggressively progressing prostate cancer (PCa) from patients with less aggressively progressing PCa, wherein the patient's body fluid concentration of human glandular kallikrein 2 (hK2) has been determined, <u>characterized</u> in that hK2 alone is used as the marker.

6. The method according to claim 5 wherein the patient's body fluid concentration of prostate specific antigen (PSA) also has been determined, <u>characterized</u> in that the patients have total PSA in the range 1 to 20 ng/ml.

7. The method according to claim 6, <u>characterized</u> in that the patients have total PSA in the range 3 to 15 ng/ml.

8. The method according to claim 5, 6 or 7, characterized in that it is a discrimination of patients with well and moderately differentiated PCa on the one hand, and poorly differentiated PCa on the other hand.

9. The method according to claim 5, 6 or 7, characterized in that it is a discrimination of patients with moderately differentiated PCa on the one hand, and poorly differentiated PCa on the other hand.

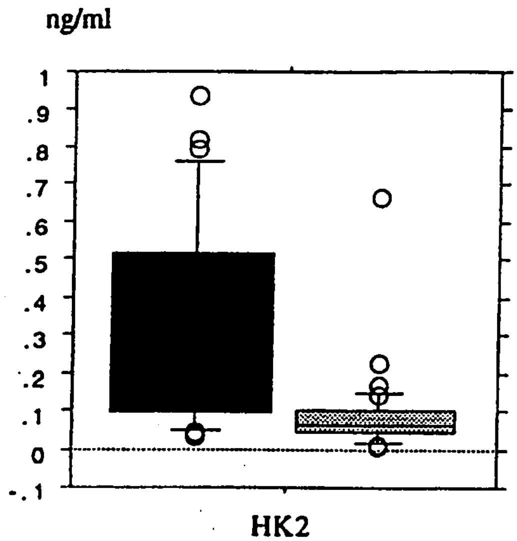

FIG. 1a

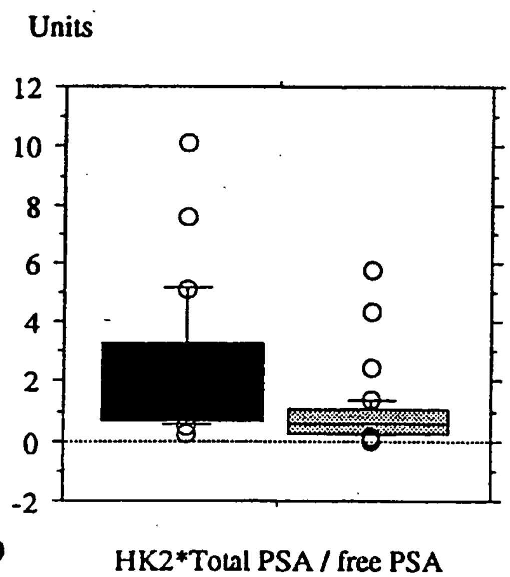

FIG. 1b

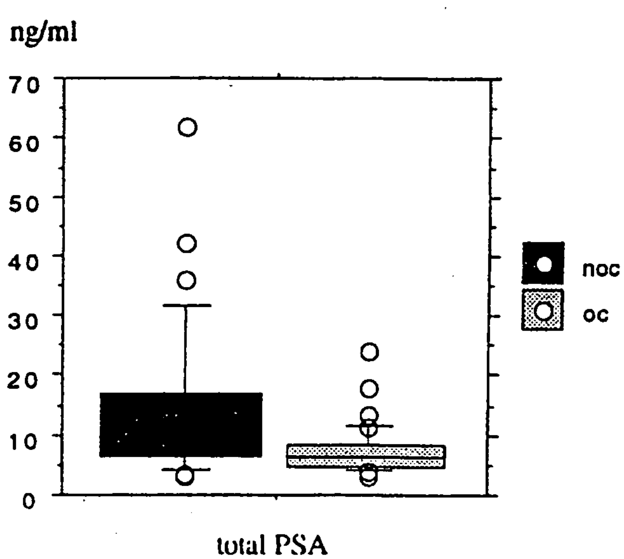

FIG. 1c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/01059

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/574
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	National Library of Medicine (NLM), file Medline, Medline accession no. 99089857, Recker F et al: "The improtance of human glandular kallikrein and its correlation with different prostate specific antigen serum forms in the detection of prostate carcinoma"; & Cancer 1998 Dec 15;83 (12):2540-7	1,3,4
Y		1-9
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X	WO 9821365 A1 (MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH), 22 May 1998 (22.05.98), see claims 99-34, 39-42 and page 7	1-9
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2000	18 -05- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/01059

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9846795 A1 (BAYLOR COLLEGE OF MEDICINE), 22 October 1998 (22.10.98), see claims 21-26, page 5, lines 20-31 --	1-9
Y	WO 9626442 A1 (LILJA, HANS), 29 August 1996 (29.08.96), see page 4-6, 14-15 and claims --	1-9
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